Video Article

Specific Marking of HIV-1 Positive Cells using a Rev-dependent Lentiviral Vector Expressing the Green Fluorescent Protein

Jia Guo*, Clinton Enos*, Yuntao Wu

National Center for Biodefense and Infectious Diseases, Department of Molecular and Microbiology, George Mason University

*These authors contributed equally

Correspondence to: Yuntao Wu at ywu8@gmu.edu

URL: http://www.jove.com/index/Details.stp?ID=2198

DOI: 10.3791/2198

Citation: Guo J., Enos C., Wu Y. (2010). Specific Marking of HIV-1 Positive Cells using a Rev-dependent Lentiviral Vector Expressing the Green Fluorescent Protein. JoVE. 43. http://www.jove.com/index/Details.stp?ID=2198, doi: 10.3791/2198

Abstract

Most of HIV-responsive expression vectors are based on the HIV promoter, the long terminal repeat (LTR). While responsive to an early HIV protein, Tat, the LTR is also responsive to cellular activation states and to the local chromatin activity where the integration has occurred. This can result in high HIV-independent activity, and has restricted the usefulness of LTR-based reporter to mark HIV positive cells [1,2,3]. Here, we constructed an expression lentiviral vector that possesses, in addition to the Tat-responsive LTR, numerous HIV DNA sequences that include the Rev-response element and HIV splicing sites [4,5,6]. The vector was incorporated into a lentiviral reporter virus, permitting highly specific detection of replicating HIV in living cell populations. The activity of the vector was measured by expression of the green fluorescence protein (GFP). The application of this vector as reported here offers a novel alternative approach to existing methods, such as *in situ* PCR or HIV antigen staining, to identify HIV-positive cells. The vector can also express therapeutic genes for basic or clinical experimentation to target HIV-positive cells.

Protocol

1. Transfection of Plasmids for Production of the Rev-dependent Lentiviral Particles

Set up: The Rev-dependent GFP lentiviral vector, pNL-GFP-RRE-SA, was described previously[4,5,6]. To assemble into a viral particle, the plasmid was contransfected into HEK293 T cells with an HIV-1 packaging construct, pCMVΔ8.2 (kindly provided by Dr. Dider Trono), and a plasmid carrying the VSV-G glycoprotein (pHCMV-G). The transfection was carried out using the calcium phosphate method.

Preparation of buffers: 10 X HBS (Hepes Buffered Saline) was prepared by dissolving 5 g of Hepes, 8 g of NaCl, 0.37 g of KCl, 1 g of Dextrose, 0.103 g of Na_2HPO_4 (anhydrous) in 100 mL of H_2O. Aliquot the10 X HBS buffer into 1 mL aliquots and store at -20°C. At the time of transfection, convert the 10 X HBS to 2 X HBS with H_2O (1: 5 dilution), and adjust the pH to between 7.05 - 7.12. (for 10 mL 2 X HBS, it usually requires approximately 50 µL of 1M NaOH). Filter sterilize the 2X HBS buffer by passing through a 0.22 µM Millipore filter. $CaCl_2$ buffer was made by dissolving $CaCl_2$ in 10 mM Hepes to a final concentration 2M. Adjust pH to 5.8, filter sterilize the buffer and store at 4°C.

Procedure:

1. One day prior to transfection, seed 2 x 10^6 HEK293T cells in a 100 mm Petri-dish, and grow cells overnight in 10 mL DMEM + 10% FBS at 37°C, 5% CO_2.
2. The next day, remove supernatant from cells and replace with 5 mL fresh DMEM + 10% FBS, continuously culture cells for 4 hours.
3. In a 5 mL polystyrene tube, add 480 µL 2X HBS buffer.
4. In a second 5 mL polystyrene tube, add 60 µL 2M $CaCl_2$ buffer, the plasmid DNA, and transfection TE buffer (1 mM Tris, 25 mM EDTA, pH 8.0) to a total volume of 480 µL. For each Petri-dish, plasmids should be added in the ratio of 10 µg of pNL-GFP-RRE-SA, 7.5 µg of pCMVΔR8.3, and 2.5 mg of pHCMV-G.
5. Add the plasmid DNA-$CaCl_2$ mixture dropwise to the 2 X HBS tube, and incubate at room temperature for 30 minutes. A fine precipitate will form.
6. Add the 960 µL of the mixture dropwise by pipette to the cells. Incubate at 37°C, 5% CO_2 overnight.
7. The next day, remove the supernatant and add 10 mL fresh DMEM + 10% FBS, and continuously incubate the cells at 37°C, 5% CO_2 overnight.
8. At 48 hours post transfection, harvest the virus by removing the supernatant and transferring it into 50 mL sterile tubes. Add fresh 10 mL fresh DMEM + 10% FBS into each Petri-dish and continue to culture overnight. Store the virus supernatant at 4°C.
9. Continue to harvest at 72 hours post transfection by collecting the supernatant. Combine all the supernatants, and centrifuge the supernatants at 500 x g for 15 minutes to pellet and remove cell debris.
10. The supernatant were collected and filtered through a 0.22 µM Millipore filter. The virus was further concentrated through size-exclusion columns.

2. Concentrate Viral Particles and Determine Viral Titer

1. Viral particles were concentrated by a size-exclusion column (100,000 MW cut off, Centricoin). Viral supernatant was loaded into the column, and centrifuged at 6,000 x g at 4°C for 15 minutes.
2. Concentrated viral supernatant was collected, aliquoted, and stored at -80°C.
3. The viral titer was determined by infection of a TNF-treated, HIV-1 positive Jacket cell line, J1.1 7. Cells were cultured in a 96 well plate at 2.5 x 10^5 cells per mL (100 mL total volume), and infected with 100 mL of serially diluted vNL-GFP-RRE-SA for a week. The titer (TCID50) of the particle was estimated by counting the GFP positive wells following the method of Reed and Muench 8.

3. Marking HIV-1 Positive Cells with the Lentiviral Particle, vNL-GFP-RRE-SA

Set up: a human CD4 T cell line, CEM-SS, was first infected with HIV-1. At 48 hours post infection, cells were superinfected with the lentiviral particles, vNL-GFP-RRE-SA. Following superinfection for another two days, GFP-positive cells were analyzed by flow cytometry. As a control, HIV-1 uninfected CEM-SS cells were identically infected with vNL-GFP-RRE-SA. Only the HIV-1-infected CEM-SS cells gave rise to GFP positive cells but not the HIV-1 uninfected CEM-SS cells.

Procedure:

1. Two hours before infection, add polybrane to a final concentration 4 mg / mL. Count cells and take 2 x 10^5 cells for each infection. Infect cells with 200 ng (p24) of HIV-1_{NL4-3} for 2 hours.
2. Washing cells by centrifuge at 300 x g for 10 minutes, resuspend cells into 2 x 10^5 cells / mL, and culture at 37°C for 48 hours.
3. Count cells and take 2 x 10^5 cells per infection with vNL-GFP-RRE-SA. Add 100 mL of vNL-GFP-RRE-SA and incubate at 37°C overnight. Wash cells and resuspend into 2 x 10^5 cells / mL.
4. Perform flow cytometry analysis at 48 hours post superinfection. Infected cells were pelleted and resuspended into 500 mL 1% paraformaldehede, incubated at room temperature for 20 minutes, and then analyzed on a FACScan analyzer (Becton Dickenson).

4. Representative Results

If the experiments are successfully performed, a sizable GFP population will be detected in HIV-1-infected CEM-SS cells by the flow cytometer, whereas, in the control, GFP positive cells will not be detected in HIV-1 uninfected CEM-SS cells.

If the experiments are successfully performed, the Rev-dependent lentiviral vector, vNL-GFP-RRE-SA, will permit the highly specific detection of replicating HIV in living cell populations, through measurement of green fluorescence protein (GFP) expression. In this example, harvested CEM-SS cells were stained with a PE-labeled rat monoclonal antibody against mouse CD24, HSA, and then analyzed on a flow cytometer for both HSA and GFP expression.

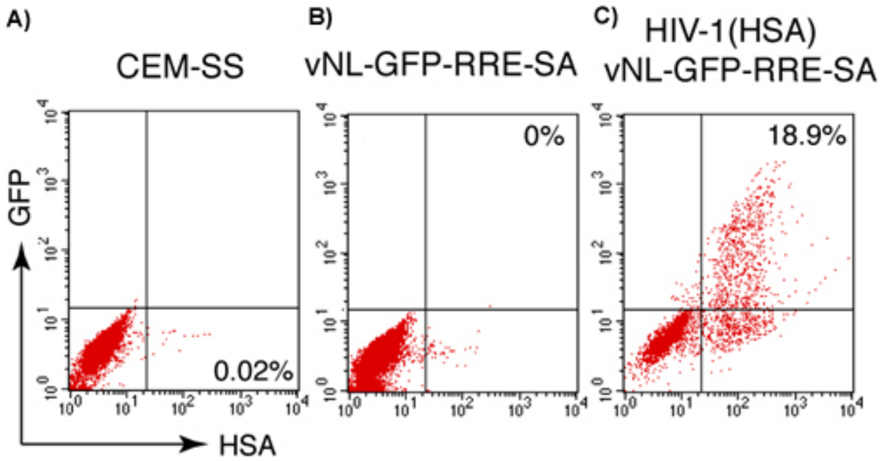

Figure 1. As shown here, a sizable GFP population was detected in HIV-1-infected cells superinfected with vNL-GFP-RRE-SA (Figure 1c). In contrast, GFP positive cells were not detected in either HIV-1 uninfected CEM-SS cells without lentiviral superinfection (Figure 1a) or HIV-1 uninfected cells with lentiviral superinfection (Figure 1b).

Disclosures

No conflicts of interest declared.

Discussion

As with the earlier developed Tat-dependent expression vectors, the Rev system described here is an exploitation of an evolved HIV process. The inclusion of Rev-dependency renders the LTR-based expression vector highly dependent on the presence of replicating HIV. The application of this vector as reported here, an HIV-dependent reporter virus, offers a novel new approach to identify HIV-positive cells. The vector permits examination of living cells, can express any gene for basic or clinical experimentation, and as a pseudo-typed lentivirus has access to most cell types and tissues.

Acknowledgements

The work was supported in part by Public Health Service grant 1R01AI081568 from NIAID to Y. W. and by the generous donations of the donors and riders of the 2009 NYCDC AIDS Ride organized by M. Rosen and Day2 Inc.

References

1. Garcia, J. A., Wu, F. K., Mitsuyasu, R. & Gaynor, R. B. Interactions of cellular proteins involved in the transcriptional regulation of the human immunodeficiency virus. *EMBO Journal* 6, 3761-3770 (1987).
2. Merzouki, *A. et al.* HIV-1 gp120/160 expressing cells upregulate HIV-1 LTR directed gene expression in a cell line transfected with HIV-1 LTR-reporter gene constructs. *Cell Mol Biol* (Noisy-le-grand) 41, 445-452 (1995).

3. Aguilar-Cordova, E., Chinen, J., Donehower, L., Lewis, D. E. & Belmont, J. W. A sensitive reporter cell line for HIV-1 tat activity, HIV-1 inhibitors, and T cell activation effects. *AIDS Res Hum Retroviruses* 10, 295-301 (1994).
4. Wu, Y., Beddall, M. H. & Marsh, J. W. Rev-dependent lentiviral expression vector. *Retrovirology* 4, 12 (2007).
5. Wu, Y., Beddall, M. H. & Marsh, J. W. Rev-dependent indicator T cell line. *Current HIV Research* 5, 395-403 (2007).
6. Young, J., Tang, Z., Yu, Q., Yu, D. & Wu, Y. Selective killing of HIV-1-positive macrophages and T Cells by the Rev-dependent lentivirus carrying anthrolysin O from *Bacillus* anthracis. *Retrovirology* 5, 36 (2008).
7. Perez, V. *L. et al.* An HIV-1-infected T cell clone defective in IL-2 production and Ca^{2+} mobilization after CD3 stimulation. *Journal of Immunology* 147, 3145-3148 (1991).
8. Reed, L. J. & Muench, H. A simple method of estimating fifty percent endpoints. *American Journal of Hygiene*, 27, 493-497 (1938).

